

SEQUENCE LISTING



<110> NAKAI, JUNICHI

<120> METHOD FOR PRODUCING A BIOSENSOR PROTEIN CAPABLE OF REGULATING A
FLUORESCENCE PROPERTY OF GREEN FLUORESCENT PROTEIN, AND THE BIOSENSOR PROTEIN
PRODUCED BY THE METHOD

<130> 216339US0

<140> 09/989,025

<141> 2001-11-21

<150> JP/2000-356047

<151> 2000-11-22

<160> 17

<170> PatentIn version 3.1

<210> 1

<211> 717

<212> DNA

<213> Aequorea victoria

<220>

<221> CDS

<222> (1)..(717)

<223>

<400> 1

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1			5						10					15		
gag	ctg	gac	ggc	gac	gta	aac	ggc	cac	aag	ttc	agc	gtg	tcc	ggc	gag	96
Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	
			20					25					30			
ggc	gag	ggc	gat	gcc	acc	tac	ggc	aag	ctg	acc	ctg	aag	ttc	atc	tgc	144
Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	
			35				40					45				
acc	acc	ggc	aag	ctg	ccc	gtg	ccc	tgg	ccc	acc	ctc	gtg	acc	acc	ctg	192
Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	
	50					55					60					
acc	tac	ggc	gtg	cag	tgc	ttc	agc	cgc	tac	ccc	gac	cac	atg	aag	cag	240
Thr	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	
65				70					75						80	
cac	gac	ttc	ttc	aag	tcc	gcc	atg	ccc	gaa	ggc	tac	gtc	cag	gag	cgc	288
His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	
				85					90					95		
acc	atc	ttc	ttc	aag	gac	gac	ggc	aac	tac	aag	acc	cgc	gcc	gag	gtg	336
Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	
			100					105					110			
aag	ttc	gag	ggc	gac	acc	ctg	gtg	aac	cgc	atc	gag	ctg	aag	ggc	atc	384
Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	
		115				120						125				
gac	ttc	aag	gag	gac	ggc	aac	atc	ctg	ggg	cac	aag	ctg	gag	tac	aac	432
Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	
	130					135					140					
tac	aac	agc	cac	aac	gtc	tat	atc	atg	gcc	gac	aag	cag	aag	aac	ggc	480
Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn	Gly	
145				150					155						160	
atc	aag	gtg	aac	ttc	aag	atc	cgc	cac	aac	atc	gag	gac	ggc	agc	gtg	528
Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser	Val	
			165						170					175		
cag	ctc	gcc	gac	cac	tac	cag	cag	aac	acc	ccc	atc	ggc	gac	ggc	ccc	576
Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	
			180					185					190			

gtg	ctg	ctg	ccc	gac	aac	cac	tac	ctg	agc	acc	cag	tcc	gcc	ctg	agc	624
Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	
	195						200					205				

aaa	gac	ccc	aac	gag	aag	cgc	gat	cac	atg	gtc	ctg	ctg	gag	ttc	gtg	672
Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe	Val	
	210					215					220					

acc	gcc	gcc	ggg	atc	act	ctc	ggc	atg	gac	gag	ctg	tac	aag	taa		717
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<210> 2

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<212> PRT

<213> Aequorea victoria

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Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu
			20					25					30		

Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys
		35					40					45			

Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu
	50					55					60				

Thr	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln
65					70					75					80

His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg
				85					90					95	

Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val
			100					105					110		

Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115 120 125

Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130 135 140

Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145 150 155 160

Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
165 170 175

Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180 185 190

Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195 200 205

Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
210 215 220

Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
225 230 235

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<211> 447

<212> DNA

<213> Rattus norvegicus

<220>

<221> CDS

<222> (1)..(444)

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Ala Asp Gln Leu Thr Glu Glu Gln Ile Ala Glu Phe Lys Glu Ala Phe	
1 5 10 15	
tcc cta ttt gac aag gac ggg gat ggg aca ata aca acc aag gag ctg	96
Ser Leu Phe Asp Lys Asp Gly Asp Gly Thr Ile Thr Thr Lys Glu Leu	
20 25 30	
ggg acg gtg atg cgg tct ctg ggg cag aac ccc aca gaa gca gag ctg	144
Gly Thr Val Met Arg Ser Leu Gly Gln Asn Pro Thr Glu Ala Glu Leu	
35 40 45	
cag gac atg atc aat gaa gta gat gcc gac ggt aat ggc aca atc gac	192
Gln Asp Met Ile Asn Glu Val Asp Ala Asp Gly Asn Gly Thr Ile Asp	
50 55 60	
ttc cct gaa ttc ctg aca atg atg gca aga aaa atg aaa gac aca gac	240
Phe Pro Glu Phe Leu Thr Met Met Ala Arg Lys Met Lys Asp Thr Asp	
65 70 75 80	
agt gaa gaa gaa att aga gaa gcg ttc cgt gtg ttt gat aag gat ggc	288
Ser Glu Glu Glu Ile Arg Glu Ala Phe Arg Val Phe Asp Lys Asp Gly	
85 90 95	
aat ggc tac atc agt gca gca gag ctt cgc cac gtg atg aca aac ctt	336
Asn Gly Tyr Ile Ser Ala Ala Glu Leu Arg His Val Met Thr Asn Leu	
100 105 110	
gga gag aag tta aca gat gaa gag gtt gat gaa atg atc agg gaa gca	384
Gly Glu Lys Leu Thr Asp Glu Glu Val Asp Glu Met Ile Arg Glu Ala	
115 120 125	
gac atc gat ggg gat ggt cag gta aac tac gaa gag ttt gta caa atg	432
Asp Ile Asp Gly Asp Gly Gln Val Asn Tyr Glu Glu Phe Val Gln Met	
130 135 140	
atg aca gcg aag tga	447
Met Thr Ala Lys	
145	

<210> 4

<211> 148

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<213> Rattus norvegicus

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Ala Asp Gln Leu Thr Glu Glu Gln Ile Ala Glu Phe Lys Glu Ala Phe
1 5 10 15

Ser Leu Phe Asp Lys Asp Gly Asp Gly Thr Ile Thr Thr Lys Glu Leu
20 25 30

Gly Thr Val Met Arg Ser Leu Gly Gln Asn Pro Thr Glu Ala Glu Leu
35 40 45

Gln Asp Met Ile Asn Glu Val Asp Ala Asp Gly Asn Gly Thr Ile Asp
50 55 60

Phe Pro Glu Phe Leu Thr Met Met Ala Arg Lys Met Lys Asp Thr Asp
65 70 75 80

Ser Glu Glu Glu Ile Arg Glu Ala Phe Arg Val Phe Asp Lys Asp Gly
85 90 95

Asn Gly Tyr Ile Ser Ala Ala Glu Leu Arg His Val Met Thr Asn Leu
100 105 110

Gly Glu Lys Leu Thr Asp Glu Glu Val Asp Glu Met Ile Arg Glu Ala
115 120 125

Asp Ile Asp Gly Asp Gly Gln Val Asn Tyr Glu Glu Phe Val Gln Met
130 135 140

Met Thr Ala Lys
145

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<211> 63

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<213> Artificial Sequence

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<222> (1)..(63)

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Ser	Ser	Arg	Arg	Lys	Trp	Asn	Lys	Thr	Gly	His	Ala	Val	Arg	Ala	Ile	
1				5					10					15		

ggt	cgg	ctg	agc	tca												63
Gly	Arg	Leu	Ser	Ser												
				20												

<210> 6

<211> 21

<212> PRT

<213> Artificial Sequence

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<223> Synthetic DNA

<400> 6

Ser	Ser	Arg	Arg	Lys	Trp	Asn	Lys	Thr	Gly	His	Ala	Val	Arg	Ala	Ile
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Gly	Arg	Leu	Ser	Ser											
				20											

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<211> 1251

<212> DNA

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<222> (1)..(1248)

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1				5					10					15		

aga	gct	ata	ggt	cgg	ctg	agc	tca	ctc	gag	aac	gtc	tat	atc	atg	gcc	96
Arg	Ala	Ile	Gly	Arg	Leu	Ser	Ser	Leu	Glu	Asn	Val	Tyr	Ile	Met	Ala	
			20					25					30			

gac	aag	cag	aag	aac	ggc	atc	aag	gtg	aac	ttc	aag	atc	cgc	cac	aac	144
Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	
		35					40					45				

atc	gag	gac	ggc	agc	gtg	cag	ctc	gcc	gac	cac	tac	cag	cag	aac	acc	192
Ile	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	
	50					55					60					

ccc	atc	ggc	gac	ggc	ccc	gtg	ctg	ctg	ccc	gac	aac	cac	tac	ctg	agc	240
Pro	Ile	Gly	Asp	Gly	Pro	Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	
65					70					75					80	

acc	cag	tcc	gcc	ctg	agc	aaa	gac	ccc	aac	gag	aag	cgc	gat	cac	atg	288
Thr	Gln	Ser	Ala	Leu	Ser	Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	
				85					90					95		

gtc	ctg	ctg	gag	ttc	gtg	acc	gcc	gcc	ggg	atc	act	ctc	ggc	atg	gac	336
Val	Leu	Leu	Glu	Phe	Val	Thr	Ala	Ala	Gly	Ile	Thr	Leu	Gly	Met	Asp	
			100					105					110			

gag	ctg	tac	aag	ggc	ggt	acc	gga	ggg	agc	atg	gtg	agc	aag	ggc	gag	384
Glu	Leu	Tyr	Lys	Gly	Gly	Thr	Gly	Gly	Ser	Met	Val	Ser	Lys	Gly	Glu	
		115					120					125				

gag	ctg	ttc	acc	ggg	gtg	gtg	ccc	atc	ctg	gtc	gag	ctg	gac	ggc	gac	432
Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	Glu	Leu	Asp	Gly	Asp	
	130					135					140					
gta	aac	ggc	cac	aag	ttc	agc	gtg	tcc	ggc	gag	ggc	gag	ggc	gat	gcc	480
Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	Gly	Glu	Gly	Asp	Ala	
	145				150					155					160	
acc	tac	ggc	aag	ctg	acc	ctg	aag	ttc	atc	tgc	acc	acc	ggc	aag	ctg	528
Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	Thr	Thr	Gly	Lys	Leu	
				165					170					175		
ccc	gtg	ccc	tgg	ccc	acc	ctc	gtg	acc	acc	ctg	acc	tac	ggc	gtg	cag	576
Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	Thr	Tyr	Gly	Val	Gln	
			180					185					190			
tgc	ttc	agc	cgc	tac	ccc	gac	cac	atg	aag	cag	cac	gac	ttc	ttc	aag	624
Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Gln	His	Asp	Phe	Phe	Lys	
		195				200					205					
tcc	gcc	atg	ccc	gaa	ggc	tac	gtc	cag	gag	cgc	acc	atc	ttc	ttc	aag	672
Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	Thr	Ile	Phe	Phe	Lys	
	210					215					220					
gac	gac	ggc	aac	tac	aag	acc	cgc	gcc	gag	gtg	aag	ttc	gag	ggc	gac	720
Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	Lys	Phe	Glu	Gly	Asp	
	225				230					235					240	
acc	ctg	gtg	aac	cgc	atc	gag	ctg	aag	ggc	atc	gac	ttc	aag	gag	gac	768
Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	Asp	Phe	Lys	Glu	Asp	
				245					250					255		
ggc	aac	atc	ctg	ggg	cac	aag	ctg	gag	tac	aac	acg	cgt	gac	caa	ctg	816
Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	Thr	Arg	Asp	Gln	Leu	
			260					265					270			
act	gaa	gag	cag	atc	gca	gaa	ttc	aaa	gaa	gct	ttc	tcc	cta	ttt	gac	864
Thr	Glu	Glu	Gln	Ile	Ala	Glu	Phe	Lys	Glu	Ala	Phe	Ser	Leu	Phe	Asp	
		275					280					285				
aag	gac	ggg	gat	ggg	aca	ata	aca	acc	aag	gag	ctg	ggg	acg	gtg	atg	912
Lys	Asp	Gly	Asp	Gly	Thr	Ile	Thr	Thr	Lys	Glu	Leu	Gly	Thr	Val	Met	
	290					295					300					
cgg	tct	ctg	ggg	cag	aac	ccc	aca	gaa	gca	gag	ctg	cag	gac	atg	atc	960
Arg	Ser	Leu	Gly	Gln	Asn	Pro	Thr	Glu	Ala	Glu	Leu	Gln	Asp	Met	Ile	
	305				310					315					320	
aat	gaa	gta	gat	gcc	gac	ggt	aat	ggc	aca	atc	gac	ttc	cct	gaa	ttc	1008
Asn	Glu	Val	Asp	Ala	Asp	Gly	Asn	Gly	Thr	Ile	Asp	Phe	Pro	Glu	Phe	
				325					330					335		

ctg	aca	atg	atg	gca	aga	aaa	atg	aaa	gac	aca	gac	agt	gaa	gaa	gaa	1056
Leu	Thr	Met	Met	Ala	Arg	Lys	Met	Lys	Asp	Thr	Asp	Ser	Glu	Glu	Glu	
			340					345					350			

att	aga	gaa	gcg	ttc	cgt	gtg	ttt	gat	aag	gat	ggc	aat	ggc	tac	atc	1104
Ile	Arg	Glu	Ala	Phe	Arg	Val	Phe	Asp	Lys	Asp	Gly	Asn	Gly	Tyr	Ile	
		355					360					365				

agt	gca	gca	gag	ctt	cgc	cac	gtg	atg	aca	aac	ctt	gga	gag	aag	tta	1152
Ser	Ala	Ala	Glu	Leu	Arg	His	Val	Met	Thr	Asn	Leu	Gly	Glu	Lys	Leu	
		370				375					380					

aca	gat	gaa	gag	gtt	gat	gaa	atg	atc	agg	gaa	gca	gac	atc	gat	ggg	1200
Thr	Asp	Glu	Glu	Val	Asp	Glu	Met	Ile	Arg	Glu	Ala	Asp	Ile	Asp	Gly	
					390					395					400	

gat	ggt	cag	gta	aac	tac	gaa	gag	ttt	gta	caa	atg	atg	aca	gcg	aag	1248
Asp	Gly	Gln	Val	Asn	Tyr	Glu	Glu	Phe	Val	Gln	Met	Met	Thr	Ala	Lys	
				405					410					415		

tga																1251
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<210> 8

<211> 416

<212> PRT

<213> Artificial Sequence

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Arg	Ala	Ile	Gly	Arg	Leu	Ser	Ser	Leu	Glu	Asn	Val	Tyr	Ile	Met	Ala
			20					25					30		

Asp	Lys	Gln	Lys	Asn	Gly	Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn
		35					40					45			

Ile	Glu	Asp	Gly	Ser	Val	Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr
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50

55

60

Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser
65 70 75 80

Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
85 90 95

Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp
100 105 110

Glu Leu Tyr Lys Gly Gly Thr Gly Gly Ser Met Val Ser Lys Gly Glu
115 120 125

Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp Gly Asp
130 135 140

Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala
145 150 155 160

Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu
165 170 175

Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr Gly Val Gln
180 185 190

Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp Phe Phe Lys
195 200 205

Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe Phe Lys
210 215 220

Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp
225 230 235 240

Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp
245 250 255

Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Thr Arg Asp Gln Leu

260

265

270

Thr Glu Glu Gln Ile Ala Glu Phe Lys Glu Ala Phe Ser Leu Phe Asp
 275 280 285

Lys Asp Gly Asp Gly Thr Ile Thr Thr Lys Glu Leu Gly Thr Val Met
 290 295 300

Arg Ser Leu Gly Gln Asn Pro Thr Glu Ala Glu Leu Gln Asp Met Ile
 305 310 315 320

Asn Glu Val Asp Ala Asp Gly Asn Gly Thr Ile Asp Phe Pro Glu Phe
 325 330 335

Leu Thr Met Met Ala Arg Lys Met Lys Asp Thr Asp Ser Glu Glu Glu
 340 345 350

Ile Arg Glu Ala Phe Arg Val Phe Asp Lys Asp Gly Asn Gly Tyr Ile
 355 360 365

Ser Ala Ala Glu Leu Arg His Val Met Thr Asn Leu Gly Glu Lys Leu
 370 375 380

Thr Asp Glu Glu Val Asp Glu Met Ile Arg Glu Ala Asp Ile Asp Gly
 385 390 395 400

Asp Gly Gln Val Asn Tyr Glu Glu Phe Val Gln Met Met Thr Ala Lys
 405 410 415

<210> 9

<211> 66

<212> DNA

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 gtcaga 66

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<211> 62

<212> DNA

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 tt 62

<210> 11

<211> 35

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 ggacgcgtac tagtaacgtc tatatcatgg ccgac 35

<210> 12

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<212> DNA

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<400> 12

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ggacgcgtga ccaactgact gaagagcag

29

<210> 16

<211> 34

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<213> Artificial Sequence

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<400> 16

gcgcggccgc tcacttcgct gtcattcattt gtac

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<210> 17

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<212> PRT

<213> Artificial Sequence

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<223> Synthetic Peptide

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